

Complete Set of Claims As Preliminarily Amended Pursuant to 37 C.F.R. § 1.121(c)(1)(3)

2. A method for isolating a peptide, polypeptide, or protein molecule from a sample in a vessel, comprising the steps of:
  - (a) combining the sample containing a peptide, polypeptide, or protein molecule of interest with affinity particles suitable for binding said molecule, said affinity particles being insoluble in the sample;
  - (b) collecting the affinity particles;
  - (c) separating the affinity particles from the unbound remainder of the sample;
  - (d) optionally, resuspending the affinity particles in a solution;
  - (e) optionally, eluting said molecule from the affinity particles, followed by separating the affinity particles from said eluted molecule;wherein at least one of steps (a), (b), (c), (d) if present, and (e) if present is performed in the presence of detergent sufficient to reduce loss of particles during any separation step, in comparison to the same method performed in the absence of detergent.
3. The method according to Claim 2, wherein the combining step (a) is carried out in the absence of detergent, but detergent is added prior to the separation step (b).
5. The method according to Claim 2, wherein said molecule is a fusion protein or peptide.
6. The method according to Claim 5, wherein said fusion protein is a protein or peptide fused to a metal chelating group.
7. The method according to Claim 6, wherein said metal chelating group is two or more histidine residues.
8. The method according to Claim 6, wherein said metal chelating group is six consecutive histidine residues.

13. The method according to Claim 2, wherein said particles are selected from the group consisting of ferromagnetic beads, superparamagnetic beads, and combinations thereof.
14. The method according to Claim 2, wherein said particles are composed of materials selected from the group consisting of agarose, silica, nitrocellulose, cellulose, acrylamide, latex, polystyrene, polyacrylate, polymethacrylate, polyethylene polymers, glass particles, silicates, metal oxides, apatites, and combinations thereof.
15. The method according to Claim 14, wherein said particles are coated with an affinity ligand selected from the group consisting of antibodies for a particular antigen, antigens for a particular antibody, antibodies recognizing a class of molecules, streptavidin, streptavidin-tagged fusion proteins, biotin, biotin-tagged fusion proteins, glutathione, cellulose, amylose, ion exchange groups, hydrophobic interaction groups, binding molecules for cell-surface markers, phage ligands, antibodies recognizing cell or phage surface antigens, and polypeptides, nucleotides or small molecules capable of affinity interactions with a binding partner selected from the group consisting of peptides, polypeptides, and proteins.
16. The method according to Claim 2, wherein said detergent, where present, is at a concentration of from about 0.0005% to 2.0% (v/v).
17. The method according to Claim 16, wherein said detergent is selected from a group consisting of nonionic detergents, anionic detergents, zwitterionic detergents, cationic detergents, and combinations thereof.
18. The method according to Claim 17, wherein said nonionic detergent is selected from the group consisting of polyoxyethylene (10) cetyl alcohol, polyoxyethylene (20) cetyl alcohol, polyoxyethylene (23) lauryl alcohol, polyoxyethylene (4-5) *p-t*-octyl phenol, polyoxyethylene (7-8) *p-t*-octyl phenol, polyoxyethylene (9) *p-t*-octyl phenol, polyoxyethylene (9-10) *p-t*-octyl phenol, polyoxyethylene (9-10) nonylphenol,

polyoxyethylene (20) sorbitol monolaurate, polyoxyethylene (20) sorbitol monopalmitate, polyoxyethylene (20) sorbitol monooleate, octyl- $\beta$ -glucoside, APO-10, APO-12, cyclohexyl-*n*-ethyl- $\beta$ -D-maltoside, cyclohexyl-*n*-hexyl- $\beta$ -D-maltoside, cyclohexyl-*n*-methyl- $\beta$ -maltoside, *n*-decanoylsucrose, *n*-decyl- $\beta$ -D-glucopyranoside, *n*-decyl- $\beta$ -maltopyranoside, *n*-decyl- $\beta$ -D-thiomaltoside, *n*-dodecanoyl sucrose, and heptane-1,2,3-triol, and combinations thereof.

19. The method according to Claim 17, wherein said nonionic detergent is polyoxyethylene (20) sorbitol monolaurate.
20. The method according to Claim 17, wherein said anionic detergent is selected from the group consisting of sodium dodecyl sulfate (SDS), sarkosyl, and combinations thereof.
21. The method Claim 17, wherein said zwitterionic detergent is 3-[(cholamido-propyl)-dimethyl-ammonio]-1-propanesulfonate.
22. The method according to Claim 17, wherein said cationic detergent dodecyl-trimethyl ammonium chloride.
23. The method according to Claim 2, wherein the detergent, where present, is a nonionic detergent at a concentration of at least about 0.005% (v/v).
24. The method according to Claim 2, wherein the detergent, where present, is a nonionic detergent at a concentration not exceeding about 2% (v/v).
25. The method according to Claim 2, wherein the detergent, where present, is an anionic detergent at a concentration of at least about 0.05% (v/v).
26. The method according to Claim 2, wherein the detergent, where present, is an anionic detergent at a concentration not exceeding about 1% (v/v).

27. The method according to Claim 2, wherein the detergent, where present, is a cationic detergent at a concentration of at least about 0.5% (v/v).
28. The method according to Claim 2, wherein the detergent, where present, is a cationic detergent at a concentration not exceeding about 1% (v/v).
29. The method according to Claim 2, wherein the detergent, where present, is a zwitterionic detergent at a concentration of at least about 0.01% (v/v).
30. The method according to Claim 2, wherein the detergent, where present, is a zwitterionic detergent at a concentration not exceeding about 2% (v/v).
32. The method according to Claim 2, wherein the molecule is a protein, polypeptide, or peptide and the detergent is polyoxyethylene (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).
34. A method for isolating a peptide, polypeptide, or protein molecule from a sample in a vessel, comprising the steps of:
  - (a) providing a multiplicity of affinity particles and incubating said particles in the presence of a detergent;
  - (b) combining the sample containing a peptide, polypeptide, or protein molecule of interest with affinity particles suitable for binding said molecule, said affinity particles being insoluble in the sample;
  - (c) collecting the affinity particles;
  - (d) separating the affinity particles from the unbound remainder of the sample;
  - (e) optionally, resuspending the affinity particles in a solution;
  - (f) optionally, eluting said molecule from the affinity particles, followed by separating the affinity particles from said eluted molecule;wherein any of the steps (b), (c), (d), (e) if present, and (f) if present may optionally be also performed in the presence of detergent, wherein the use of detergent is sufficient to

reduce loss of particles during any separation step, in comparison to the same method performed in the absence of detergent.

36. The method according to Claim 34, wherein said molecule is a fusion protein or peptide.
37. The method according to Claim 36, wherein said fusion protein is a protein or peptide fused to a metal chelating group.
38. The method according to Claim 37, wherein said metal chelating group is two or more histidine residues.
39. The method according to Claim 37, wherein said metal chelating group is six consecutive histidine residues.
44. The method according to Claim 34, wherein said particles are selected from the group consisting of ferromagnetic beads, superparamagnetic beads, and combinations thereof.
45. The method according to Claim 34, wherein said particles are composed of materials selected from the group consisting of agarose, silica, nitrocellulose, cellulose, acrylamide, latex, polystyrene, polyacrylate, polymethacrylate, polyethylene polymers, glass particles, silicates, metal oxides, apatites, combinations thereof.
46. The method according to Claim 45, wherein said particles are coated with an affinity ligand selected from the group consisting of antibodies for a particular antigen, antigens for a particular antibody, antibodies recognizing a class of molecules, streptavidin, streptavidin-tagged fusion proteins, biotin, biotin-tagged fusion proteins, glutathione, cellulose, amylose, ion exchange groups, hydrophobic interaction groups, binding molecules for cell-surface markers, phage ligands, antibodies recognizing cell or phage surface antigens, and polypeptides capable of affinity interactions with a binding partner selected from the group consisting of peptides, polypeptides, and proteins.

47. The method according to Claim 34, wherein said detergent, where present, is at a concentration of from about 0.0005% to 2.0% (v/v).
48. The method according to Claim 47, wherein said detergent is selected from a group consisting of nonionic detergents, anionic detergents, zwitterionic detergents, cationic detergents, and combinations thereof.
49. The method according to Claim 48, wherein said nonionic detergent is selected from the group consisting of polyoxyethylene (10) cetyl alcohol, polyoxyethylene (20) cetyl alcohol, polyoxyethylene (23) lauryl alcohol, polyoxyethylene (4-5) *p*-*t*-octyl phenol, polyoxyethylene (7-8) *p*-*t*-octyl phenol, polyoxyethylene (9) *p*-*t*-octyl phenol, polyoxyethylene (9-10) *p*-*t*-octyl phenol, polyoxyethylene (9-10) nonylphenol, polyoxyethylene (20) sorbitol monolaurate, polyoxyethylene (20) sorbitol monopalmitate, polyoxyethylene (20) sorbitol monooleate, octyl- $\beta$ -glucoside, APO-10, APO-12, cyclohexyl-*n*-ethyl- $\beta$ -D-maltoside, cyclohexyl-*n*-hexyl- $\beta$ -D-maltoside, cyclohexyl-*n*-methyl- $\beta$ -maltoside, *n*-decanoylsucrose, *n*-decyl- $\beta$ -D-glucopyranoside, *n*-decyl- $\beta$ -maltopyranoside, *n*-decyl- $\beta$ -D-thiomaltoside, *n*-dodecanoyl sucrose, and heptane-1,2,3-triol, and combinations thereof.
50. The method according to Claim 49, wherein said nonionic detergent is polyoxyethylene (20) sorbitol monolaurate.
51. The method according to Claim 48, wherein said anionic detergent is selected from the group consisting of sodium dodecyl sulfate (SDS), sarkosyl, and combinations thereof.
52. The method Claim 48, wherein said zwitterionic detergent is 3-[(cholamido-propyl)-dimethyl-ammonio]-1-propanesulfonate.
53. The method according to Claim 48, wherein said cationic detergent dodecyl-trimethyl ammonium chloride.

54. The method according to Claim 34, wherein the detergent, where present, is a nonionic detergent at a concentration of at least about 0.005% (v/v).
55. The method according to Claim 34, wherein the detergent, where present, is a nonionic detergent at a concentration not exceeding about 2% (v/v).
56. The method according to Claim 34, wherein the detergent, where present, is an anionic detergent at a concentration of at least about 0.05% (v/v).
57. The method according to Claim 34, wherein the detergent, where present, is an anionic detergent at a concentration not exceeding about 1% (v/v).
58. The method according to Claim 34, wherein the detergent, where present, is a cationic detergent at a concentration of at least about 0.5% (v/v).
59. The method according to Claim 34, wherein the detergent, where present, is a cationic detergent at a concentration not exceeding about 1% (v/v).
60. The method according to Claim 34, wherein the detergent, where present, is a zwitterionic detergent at a concentration of at least about 0.01% (v/v).
61. The method according to Claim 34, wherein the detergent, where present, is a zwitterionic detergent at a concentration not exceeding about 2% (v/v).
62. The method according to Claim 34, wherein the molecule is a nucleic acid and the detergent is polyoxyethylene (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).

63. The method according to Claim 34, wherein the molecule is a protein or peptide and the detergent is polyoxyethylene (20) sorbitol monolaurate at a concentration of at least about 0.005% (v/v).
64. A method for isolating a peptide, polypeptide, or protein molecule from a sample in a vessel, comprising the steps of:
  - (a) combining the sample containing a peptide, polypeptide, or protein molecule of interest with magnetic affinity particles suitable for binding said molecule, said magnetic affinity particles being insoluble in the sample;
  - (b) applying a magnetic field to the vessel so as to attract and immobilize the magnetic affinity particles;
  - (c) separating the unimmobilized remainder of the sample from the immobilized magnetic affinity particles;
  - (d) optionally, resuspending the magnetic affinity particles in a solution;
  - (e) optionally, eluting said molecule from the affinity particles, followed by separating the affinity particles from said eluted molecule;wherein at least one of steps (a), (b), (c), (d) if present, and (e) if present is performed in the presence of detergent sufficient to reduce loss of particles during any separation step, in comparison to the same method performed in the absence of detergent.
65. The method according to Claim 64, wherein the combining step (a) is carried out in the absence of detergent, but detergent is added prior to the application of a magnetic field in accordance with step (b).
66. A method for isolating a peptide, polypeptide, or protein molecule from a sample in a vessel, comprising the steps of:
  - (a) providing a multiplicity of magnetic affinity particles and incubating said particles in the presence of a detergent;
  - (b) combining the sample containing a peptide, polypeptide, or protein molecule of interest with said affinity particles suitable for binding said molecule, said affinity particles being insoluble in the sample;

(c) immobilizing the magnetic affinity particles by applying a magnet to said vessel;  
(d) separating the remainder of the sample from the immobilized magnetic affinity particles;  
(e) optionally, resuspending the affinity particles in a solution;  
(f) optionally, eluting said molecule from the affinity particles, followed by separating the affinity particles from said eluted molecule;  
wherein any of the steps (b), (c), (d), (e) if present, and (f) if present may optionally be also performed in the presence of detergent, wherein the use of detergent is sufficient to reduce loss of particles during any separation step, in comparison to the same method performed in the absence of detergent.

67. The method according to Claim 14 or 45, wherein the polyethylene polymer is a polyvinyl alcohol.
68. The method according to Claim 14 or 45, wherein the silicate is selected from the group consisting of calcium silicate, magnesium silicate, aluminum silicate, and combinations thereof.
69. The method according to Claim 14 or 45, wherein the metal oxide is selected from the group consisting of titanium oxide, tin oxide, and combinations thereof.